2012

Effects of Conductivity and Fish Grazing on Alkaline Phosphatase Activity of Littoral Periphyton

Samuel A. Drerup
Wright State University

EFFECTS OF CONDUCTIVITY AND FISH GRAZING ON ALKALINE PHOSPHATASE ACTIVITY OF LITTORAL PERIPHYTON

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

By

Samuel A. Drerup
B.S., Ohio University, 2009

2012
Wright State University

WRIGHT STATE UNIVERSITY
GRADUATE SCHOOL

4/18/2012

I HEARBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Samuel A. Drerup ENTITLED Effects of Conductivity and Fish Grazing on Alkaline Phosphatase Activity of Littoral Periphyton BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS OF THE DEGREE OF Master of Science

Yvonne Vadeboncoeur, Ph.D.
Thesis Advisor

David Goldstein, Ph.D., Chair
Department of Biological Sciences
College of Science and Mathematics

Committee on
Final Examination

Don Cipollini, Ph.D.

Tom Rooney, Ph.D.

Andrew Hsu, Ph.D.
Dean, Graduate School

ABSTRACT

Drerup, Samuel A. M.Sc. Department of Biological Sciences, Wright State University, 2012. Power Effects Of Conductivity And Fish Grazing On Alkaline Phosphatase Activity Of Littoral Periphyton

.

Littoral periphyton plays an important role in whole lake primary production and is a substantial contributor to whole lake energy budgets in moderately deep oligotrophic lakes. Under conditions of nutrient deficiency, members of the periphyton community supplement the available nutrient pool through the production of intracellular and extracellular enzymes. These enzymes are used to convert bio-unavailable forms of nitrogen and phosphorous to forms usable by the periphyton community. In this paper, I will investigate how conductivity and fish grazing affect periphyton production and phosphorus acquisition in both field and laboratory experiments.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER 1 PAGE

CHAPTER 2 PAGE

LIST OF FIGURES

CHAPTER 1 PAGE

CHAPTER 2 PAGE

Introduction

Lakes can be divided into three basic zones (pelagic, profundal, and littoral) based on light availability and proximity to the shore (Wetzel 2001). The pelagic zone of a lake is where light availability is high and where photosynthesis in the water column occurs. The profundal zone is the deepwater habitat with low light availability with no primary production. The littoral zone of a lake is the near shore area where enough light reaches the bottom of the lake for primary production to occur. Studies of lake ecosystems function have largely focused on the flora and fauna of the open water (pelagic zone) habitat, neglecting contributions from the littoral zone to ecosystem processes. Without accounting for littoral contributions to the ecosystem researchers risk developing a skewed interpretation of ecosystem function especially in nutrient poor lakes.

The lack of phytoplankton biomass in nutrient poor, oligotrophic, lakes results in high light availability to littoral primary producers. Periphyton is a conglomerate of many different types of algae, fungi, and bacteria attached to a substratum and a prominent primary producer in the littoral zone. Carbon fixed through primary production by littoral periphyton contributes to both the littoral and pelagic ecosystem through organism life histories and foodweb linkages. Littoral primary production is limited by light (Steinman and McIntire 1987) and nutrient, such as nitrates and inorganic phosphorus, availability (Fairchild et al 1985).

Light availability reaching the portion of sediment in the euphotic zone is positively correlated with the percentage of whole lake primary production attributed to benthic algae (Vadeboncoeur et al 2001). Periphyton plays an important role in whole

lake primary production contributing a large portion of primary production in small oligotrophic lakes (Vander Zanden et al. 2006, Vadeboncoeur et al. 2001, Vadeboncoeur et al. 2003) and is a substantial contributor to whole lake energy budget in moderately deep oligotrophic lakes (Vadeboncoeur et al. 2008). Organisms from many different trophic levels are dependent on carbon fixed by littoral benthic primary production (Hecky and Hesslein 1995, Vander Zanden and Vadeboncoeur 2002). In conditions of abundant light, such as conditions in oligotrophic lakes, epilithic periphyton production is limited by bio-available nutrients (Fairchild et al 1985). The most important limiting nutrients in aquatic systems are phosphorus and nitrogen. Under conditions of nutrient deficiency, members of the periphyton community supplement the available nutrient pool through the production of intracellular and extracellular enzymes. These enzymes are used to convert bio-unavailable forms of nitrogen and phosphorus to forms usable by the periphyton community.

Nitrogen fixation is an energy intensive process, done by heterocystous cyanobacteria, to sequester bioavailable nitrogen from the bio-unavailable dinitrogen gas using the nitrogenase enzyme. In phytoplankton, cyanobacterial dominance of the algal community is associated with lakes experiencing eutrophic conditions. Nitrogen fixation provides a competitive advantage under high phosphorus loading (Reeders et al 1998). However, benthic nitrogen fixing cyanobacteria dominate the epilithic periphyton community when sufficient light reaches the lake bottom and can contribute significantly to benthic nitrogen availability (Howarth et al 1988). Nitrogen fixation it is not the emphasis of this study but will be investigated as it relates to phosphorus acquisition. The purpose of this study is to better understand how the periphyton community meets

its phosphorus demand in nutrient poor systems.

Phosphorus exists in two general forms in aquatic systems, inorganic and organic. Inorganic phosphorus is considered bioavailable and can readily be incorporated into periphyton biomass. Organic sources of phosphorus (i.e., phosphate groups bound to carbon molecule) cannot be assimilated directly into periphyton biomass. In oligotrophic lakes, levels of total phosphorus, inorganic and organic forms, are low (3.0 to 17.7 mg per m^3) (Wetzel 2001) and primary producers experience phosphorus limitation. Thus, the ability of periphyton to sequester phosphorus bound to organic molecules is advantageous in oligotrophic environments. An enzymatic pathway for organic phosphorus conversion is through phosphomonoesterase production. Phosphomonoesterases are a group of enzymes produced by many organisms that convert organic phosphorus compounds into useable orthophosphate compounds (Jansson et al. 1988). Phosphomonoesterases are produced to address nutrient limitation experienced by periphyton.

The conversion of organic to inorganic phosphorus compounds is a four-step reaction. The enzyme first binds to the organic phosphorus substrate. Second, an alcohol is released forming a phosphoryl-enzyme compound. Third, the phosphoryl-enzyme complex is converted to a non-covalent compound with the addition of water. Finally, the orthophosphate is released and the free enzyme is regenerated (Jansson et al. 1988). Alkaline phosphatase is a non-specific phosphomonoesterase found in both intra- and extracellular environments.

The efficiency of phosphomonoesterase can be strongly influenced by environmental conditions. The production of phosphatase is inhibited by the presence of

inorganic phosphorous, and thus, phosphatase has the highest rate of hydrolysis when inorganic phosphate is limiting (Jansson et al. 1988, Chrost and Overbeck 1987, Hernandez et al. 2002). This response to inorganic phosphorus availability has made phosphatase activity an index of P limitation (Rose and Axler 1997). Alkaline phosphatase is a metalloenzyme with two Zn^{+2} ions and one Mg^{2+} ion at each activation site (Kim and Wyckoff 1991). Enzyme activity is stimulated by large additions of calcium and magnesium and/or smaller additions of sodium, potassium, or iron (Al-Shehri 2006). Alkaline phosphatase activity is highest at pH values between 8 and 10 with the optimum at pH of 8.7 to 9 (Jansson et al. 1988). Compared to other proteins, phosphatases require a high investment of nitrogen (8-32% N) indicating that phosphatase production is dependent on nitrogen availability (Treseder and Vitousek 2001) creating a nutrient paradox in oligotrophic systems. Both environmental conditions and community composition can affect periphyton enzyme activity (nitrogenase and phosphatase). In this study, I will investigate how conductivity affects periphyton production and phosphorus acquisition.

Lake Tanganyika and Lake Tahoe are two oligotrophic lakes with similar periphyton communities dominated by nitrogen fixing cyanobacteria (*Calothrix spp*.) attached to the substratum. *Calothrix* is an eyelash shaped filamentous genus of Cyanobacteria that grows attached to surfaces and has a basal heterocyst. Heterocysts are specialized cells where nitrogen fixation occurs. The ability of *Calothrix* to dominate nutrient poor systems may be related to its high levels of the alkaline phosphatase enzyme and its ability to fix atmospheric nitrogen (Healey and Hendzel 1979, Currie and Kalff 1984, Livingstone and Whitton 1984). Organisms that have the ability to fix

nitrogen have an advantage in phosphorus acquisition in phosphorus (inorganic) poor systems (Houlton et al. 2008). Primary production is increased in nutrient deficient systems with additions of nitrogen, phosphorous, or both nitrogen and phosphorous with the largest increase in production resulting from the combination of nitrogen and phosphorous (Elser et al. 2007). Bioavailable nitrogen and phosphorus generated through enzyme activity are one possible source of additional bioavailable nutrients in Lake Tanganyika and Lake Tahoe.

Although similar in nutrient concentration and periphyton community, the systems differ in important ways. Lake Tanganyika has much higher rates of primary productivity (43-52.4 $mgCm^{-2}h^{-1}$) (O'Reilly 2006) compared to Lake Tahoe (4.96 $mgCm^{-2}h^{-1}$) (Loeb 1981). Lake Tanganyika has a much greater concentration of dissolved ions that increase conductivity and alkalinity. Climate also differs drastically between Lake Tahoe (temperate) and Lake Tanganyika (tropical). However, annual average and summer water temperatures experienced by the two lakes are similar. Despite annual temperature differences attributed to latitude, no relationship exists between periphyton primary production and latitude was detected in oligotrophic lakes (O'Reilly 2006). Lake Tanganyika also has a much higher density of grazing fish compared to Lake Tahoe. These differences between lakes may also contribute to the differences in primary productivity.

Despite low nutrient concentrations, Lake Tanganyika is one of the most efficient oligotrophic lake having high rates of primary production with low periphyton biomass (O'Reilly 2006). Although periphyton production is highly efficient, phytoplankton production is the main source of energy to the whole system as pelagic surface area

dwarfs littoral area in Lake Tanganyika (Hecky and Fee 1981). Although not significant relative to phytoplankton contributions to whole system energy dynamics, littoral periphyton production supports a highly diverse and dense population of grazing fishes (Bootsma et al. 1996). How can this high level of littoral primary production and diverse fish community persist despite severe nutrient limitation of this oligotrophic system?

High concentrations of magnesium, calcium, and zinc in Lake Tanganyika could increase the production of phosphatase, a metalloenzyme with zinc and magnesium cofactors. The availability of these ions and the alkaline nature of Lake Tanganyika could result in an increase in phosphatase production and could increase the total phosphorus pool available to the periphyton, increasing production. In this study, I will investigate the effects of conductivity on periphyton production and phosphatase activity of periphyton cultures from Lake Tanganyika and Lake Tahoe. I hypothesize that increasing the concentration of metal ions in the environment will increase alkaline phosphatase activity resulting in an increase in inorganic phosphorous availability. The increase of inorganic phosphorus availability will result in an increase in periphyton production.

Methods

I designed a set of laboratory experiments to determine the effects of specific conductance on periphyton function. Specifically, I investigated the effects of the availability of micronutrients (Ca, Mg, K, and Fe) and phosphorus source (organic versus inorganic) on periphyton alkaline phosphatase activity, primary productivity, and growth.

Periphyton Culturing and Maintenance: I cultured periphyton collected from Lake Tahoe,

U.S.A. (July 2008) and Lake Tanganyika, Tanzania (July 2009) and these cultures were used in all laboratory experiments. Periphyton was removed from littoral cobbles using steel bristled brushes from multiple cobbles and the disrupted periphyton slurry was then combined into one culture per lake.

For long term maintenance of the Lake Tahoe and Lake Tanganyika communities, I used a medium (Kruskopf and DuPressis 2006) that is suitable for mixed algal cultures (polyculture). To promote cyanobacteria dominance, I modified the media recipe by removing sources of nitrogen (see Table1). One of the goals in this experiment was to determine the effect that phosphorus source had on alkaline phosphatase activity. I added either beta-glycerophosphate disodium salt or sodium phosphate as sources of organic and inorganic phosphorus respectively (Livingstone and Whitton 1984). Conductivity and pH were adjusted using Seachem™ Cichlid Lake Salt and Lake Tanganyika Buffer. Routine culture maintenance included biweekly media changes and removal of excess periphyton from the standing stock.

For each experiment, I placed twenty-five frosted (single side) glass microscope into one of two 15L glass aquaria. The aquaria were then inoculated with cultured periphyton from Lake Tanganyika or Lake Tahoe and incubated under fluorescent lighting. I attached monofilament fishing line to the microscope slides to aid in retrieval from the experimental Erlenmeyer flasks in which they would eventually be cultured. Slides were colonized for 21 days in the aquaria in polyculture media adjusted to a conductivity of 300μS per cm and pH of 8.5. During the colonization period, periphyton from both lakes received sources of organic and inorganic phosphorus at a concentration of $1 mg*L^{-1}$ (see Table 1).

After colonization, 20 slides from each aquarium were haphazardly selected and each was placed into a 500mL Erlenmeyer flask with 300mL of media. I randomly assigned each flask one of four treatments in a two by two design. Conductivity at 620$\mu S \cdot cm^{-1}$ plus organic phosphorus (HO), conductivity at 90$\mu S \cdot cm^{-1}$plus organic phosphorus (LO), conductivity at 620$\mu S \cdot cm^{-1}$ plus inorganic phosphorus (HI), and at 90$\mu S \cdot cm^{-1}$plus organic phosphorus (LI). All treatments were maintained within a pH range of 8.5-9.0. Each of the four treatments had five replicates.

Flasks were checked daily, and changes in media volume due to evaporation were corrected with the addition of deionized water. Once a week, slides were transferred to clean, acid washed flasks containing new media. The conductivity experiment from 2010 ran for 45 days but the conductivity experiment conducted in 2011 ran for only 28

Alkaline Phosphatase Activity: I measured alkaline phosphatase activity as the production of fluorescent 4-Methylumbelliferyl (4-MU) from the non-fluorescent 4-Methylumbellifery phosphate (4-MUP) substrate (Healey and Hendzel 1979). I placed the colonized microscope slides into sterile Whirl-Pak sample bags with 40mL, enough to completely cover the slides, of media HO, LO, HI, or LI. One milliliter of liquid 4-MUP (300µM) substrate was added to each sample and slides were allowed to incubate for 40 minutes under direct light. After incubation, three 5mL aliquots were collected from each Whirl-Pak and transferred to glass cuvettes for analysis.

I measured fluorescence using the Turner Designs model 700 fluorometer equipped with the Long Wavelength UV Filter Kit (excitation 360nm and emission 440nm) (Turner Designs, Sunnyvale, California). A five second average fluorescence

was recorded and APA determined by 4-MUF concentration, correcting average fluorescence by total colonize-able surface area and incubation time.

Primary Production: I measured primary productivity using the YSI PRODO optical oxygen meter (Yellow Springs Instruments, Yellow Springs, Ohio). Individual slides were placed into 473mL Nalgene wide mouth jars filled with media (HO, LO, HI, or LI). An initial dissolved oxygen measurement was recorded for each slide and then it was incubated for 15 minutes in under direct light. At the end of the incubation, I recorded a final dissolved oxygen measurement. I calculated net primary production as the change in dissolved oxygen for each slide corrected for incubation time and colonizable surface area.

Net primary production is primary production corrected for respiration by dark incubations. I covered the Nalgene containers to block light and incubated the slides under direct light to eliminate changes in respiration due to differences in incubation temperature. Net primary productivity was calculated as gross primary productivity minus the change in oxygen from the dark incubation corrected for incubation time and colonizable surface area for each slide.

Nitrogen Fixation: I used the acetylene reduction technique (Higgins et al. 2001) to measure nitrogen fixation. Algae convert acetylene to ethylene at a rate of 4:1 compared to the fixation of dinitrogen gas. I incorporated compressed acetylene into the experimental medias (HO, LO, HI, or LI) for at least one hour to ensure an acetylene saturated solution. Slides were placed into clear plastic chambers with 60mL of experimental media and overfilled with 60mL of acetylene saturated media for a final volume of 100mL. The slides were incubated for a minimum of three hours under direct

light.

At the end of the incubation, 40mL of water was sampled from the chambers and an additional 20mL of air was drawn into the syringe to create a headspace. I equilibrated the samples for two minutes using a shaker table set at 60rpm. At the end of equilibration period, 18mL of air from the headspace was injected into 12mL evacuated vials. Samples were read on a gas chromatograph equipped with a flame ionization detector. I calculated nitrogen fixation rates as the generation of ethylene gas and corrected for incubation time, acetylene to di-nitrogen gas ratio, and colonizable surface area.

I determined chlorophyll a concentration following the EPA method 445.0. Two milligrams of dried periphyton was weighed out for each sample and placed into 15mL plastic centrifuge tubes. Lyophilized samples were extracted for 24hrs in 10mL of 90% ethanol buffered with magnesium carbonate. I transferred five milliliters of extracted sample from the 15mL centrifuge tube into a disposable glass cuvette. Chlorophyll *a* concentration was determined using a Turner Designs model 700 fluorometer, equipped with the chlorophyll optical kit for in-vitro analysis (Turner Designs, Sunnyvale California). After initial reading, the sample was acidified using 0.1N HCl and was allowed to sit for exactly 90 seconds and then read again. Chlorophyll *a* measurements were corrected for pheophytin concentration.

Statistical Analysis: I preformed all statistical analyses using the “R: A Language and Environment for Statistical Computing” software (R Development Core Team, Vienna, Austria). I will refer to the treatments as: HO (high conductivity and organic phosphorus), LO (low conductivity and organic phosphorus), HI (high conductivity and

inorganic phosphorus), and LI (low conductivity and inorganic phosphorus) from this point on. Analysis of variance was done to determine differences between alkaline phosphatase activity and algal dry mass, separated by lake origin, among conductivity, phosphorous source, and conductivity by phosphorous source in the 2010 and 2011 experiments. I conducted independent statistical analyses for each of the cultures. I log transformed the data from the 2010 experiment to meet the assumptions of the ANOVA model, but no transformation was necessary for the 2011 data. Assumptions were tested using the Bartlett's Test of Homogeneity of Variances (p-value > 0.05) and the Shapiro-Wilk Normality Test (p-value > 0.05).

Results

In both the 2010 and 2011(Figure 1) experiments, area-specific alkaline phosphatase activity for Lake Tahoe and Lake Tanganyika was significantly greater in the organic phosphorus treatment compared to the inorganic phosphorous treatment (=86.62, p-value= <0.0001). There was no significant difference in area-specific APA between high and low conductivity treatments for Lake Tahoe cultures in the 2010 experiment (p-value = >0.05) (Table 2). In the 2011 experiment, Lake Tahoe periphyton cultures had significantly higher rates of alkaline phosphatase activity in the low conductivity plus organic phosphorus source (p-value=0.02, Table 3). Cultures from Lake Tanganyika had significantly higher area-specific APA in both years (p-value=<0.05, Table 4 and Table 5) when grown in the high conductivity treatment compared with cultures grown in the low conductivity treatment (organic phosphorous treatment only).

In summary, area-specific APA in both types of periphyton cultures were

significantly affected by source of phosphorous, but only cultures from Lake Tanganyika showed a significant response to conductivity.

Periphyton dry mass was also strongly influenced by media conductivity in both experiments. In 2010 and 2011(Figure 3), cultures from Lake Tahoe (Table 6 and 7) and Lake Tanganyika (Table 8 and 9) had significantly greater dry mass in the high conductivity treatments compared to the same cultures in the low conductivity media, regardless of phosphorus source. In both experiments, periphyton biomass was nearly two times greater in high conductivity treatments compared to low conductivity treatments. However, there was no significant difference in periphyton biomass between cultures grown with organic and inorganic phosphorus source at the same conductivity.

Periphyton area-specific gross primary productivity also demonstrated responses to conductivity and phosphorous source. In both years, periphyton cultures in the low conductivity and organic phosphorous treatment had the lowest measured rates of gross primary productivity (Figure 3). In both years, gross primary productivity was highest in periphyton cultures grown in the high conductivity and inorganic phosphorous treatments. Interestingly, gross primary productivity was comparable in the high conductivity & organic phosphorous and the low conductivity & inorganic phosphorous treatments. This suggests that increasing conductivity promotes primary productivity in the presence of both organic and inorganic phosphorous.

Although significant treatment responses were observed in periphyton biomass (Tables 10, 11, 12, and 13) and area-specific APA, there was no relationship of APA to algal biomass (Figure 4) or between biomass and nitrogen fixation (Figure 5) in any treatment. Regression analysis indicates no significant relationship for Lake Tahoe

periphyton and Lake Tanganyika periphyton Similar patterns are aparent in biomass-specific APA and area-specific APA in the 2010 experiment with APA being highest in the high conductivity and organic phosphorous treatment. However the pattern was not observed in the 2011 data (Figure 6).

. Lake Tahoe periphyton had significantly higher rates of nitrogen fixation compared to Lake Tanganyika periphyton across all four treatments (Figure 7, p-value < 0.001). Lake Tahoe periphyton in the inorganic phosphorus treatment had significantly greater rate of nitrogen fixation than the periphyton grown in the organic phosphorus treatment.

Discussion

This study revealed a strong effect of conductivity and phosphorous source on periphyton cultures from both Lake Tahoe and Lake Tanganyika. The decrease in alkaline phosphatase activity in the inorganic phosphorous treatment was expected because the production of alkaline phosphatase is inhibited by the presence of inorganic sources of phosphorous (Jansson et al. 1988, Chrost and Overbeck 1987). The high alkaline phosphatase activity in the organic phosphorous treatment indicates that in both cultures phosphorous demand was being met through the production of alkaline phosphatase. The periphyton from Lake Tanganyika showed a dramatic increase in alkaline phosphatase activity at the higher conductivity, whereas periphyton from Lake Tahoe did not, suggesting lake origin differences in nutrient requirements between periphyton cultures. The result from the 2010 experiment would suggest that the increase in APA (both area-specific and biomass-specific) of Lake Tanganyika periphyton was a result of media conductivity and not because of an increase in

periphyton biomass. However, this is not supported by the 2011 data. Differences in alkaline phosphatase activity between the two experiments could also be an artifact differences in experiment duration (2010=45 days, 2011= 28 days).

The cultures in the inorganic phosphorous treatment were expected to have greater biomass due to the abundance of bioavailable phosphorous. However, periphyton from Lake Tahoe and Lake Tanganyika preformed equally well, in terms of biomass accrual, in the high conductivity treatments. This result suggests that the difference in periphyton biomass among treatments was due to the increase in conductivity. This response to additional micronutrients, regardless of phosphorous source and in the absence of supplied nitrogen source, suggests that nutrient co-limitation occurred in this laboratory study. A positive relationship exists between conductivity levels and periphyton production in oligotrophic lakes as reviewed by Hammer (1981).

Similar patterns, in productivity and biomass, emerged from both the 2010 and 2011 conductivity experiment. In both the inorganic and organic treatments, the cultures with high conductivity levels had increased rates of gross primary production and higher periphyton biomass. Gross primary productivity was highest in the HI treatments indicating that these experimental conditions were better for periphyton production compared to the other three experimental conditions. Additionally, comparing the high conductivity and inorganic phosphorous treatment to only the high conductivity and organic phosphorous treatment suggests there is a cost associated with the conversion of organic phosphorous to inorganic phosphorous. When comparing gross primary productivity in the high conductivity treatments and low conductivity

treatments, percent increases in gross primary productivity are similar in both the organic and inorganic phosphorous treatments. This suggests that although a cost is associated with organic phosphorous conversion, increased conductivity still provides a boost to periphyton production.

Lake Tahoe periphyton had much higher rates of nitrogen fixation compared to Lake Tanganyika periphyton in all treatments. Nitrogen fixation in the inorganic phosphorous and high conductivity treatments was higher compared to the other treatments in the Lake Tahoe cultures. Responses observed in alkaline phosphatase activity and nitrogenase suggests that there were differences in the periphyton cultures based on lake of origin. Generally speaking, Lake Tahoe is considered nitrogen limited and Lake Tanganyika phosphorous limited and could explain why Lake Tahoe cultures had significantly higher rates of nitrogen fixation and why Lake Tanganyika had higher alkaline phosphatase activity. Periphyton from Lake Tahoe might be specialized to deal with nitrogen limitation where as periphyton from Lake Tanganyika is better suited for phosphorous limitation. Interestingly, rates of nitrogen fixation did not increase between conductivity treatments to support higher rates of gross primary productivity.

This study demonstrates the flexibility in nutrient acquisition of periphyton, particularly by heterocystous cyanobacteria. Through symbiotic relationships with heterocystous cyanobacteria, some diatom species have the ability to fix atmospheric nitrogen (Howart et al 1988). There are few members of the periphyton community that are able to convert organic phosphorous sources and fix atmospheric nitrogen. The combination of both of these enzymes allows the heterocystous cyanobacteria in Lake Tahoe and Lake Tanganyika to dominate the periphyton composition by out competing

members of the community limited by bioavailable nutrients.

The general perception is that saltwater ecosystems tend to be limited by available nitrogen and freshwater systems are limited by available phosphorous, although evidence suggests that Lake Tahoe is nitrogen and phosphorous limited (Jassby et al 1995). However, this Liebig's law of the minimum was developed in highly controlled environments and nutrient limitation becomes complicated in natural systems (Danger et al. 2008), particularly extremely nutrient poor systems (Harpole et al. 2011). Primary production is increased in nutrient deficient systems with additions of nitrogen, phosphorous, or both nitrogen and phosphorous with the largest increase in production resulting from the combination of nitrogen and phosphorous (Elser et al. 2007). The results of this study suggest a synergistic relationship between nitrogen and phosphorous in aquatic ecosystems.

Nutrient co-limitation occurs in situations where two or more nutrients, either in combination or individually, limit metabolic processes. Many studies of aquatic and terrestrial ecosystems have demonstrated important interactions between nitrogen and phosphorous implying that both can limit a system (Harpole et al. 2011). Recent studies have also highlighted the importance of micronutrients (such as nickel, zinc, and magnesium) to algal primary production and alkaline phosphatase activity (Kathuria and Martiny 2011).

Micronutrients can aid in the uptake of macronutrients through enzymatic cofactors. Empirical evidence has suggested the importance of micronutrients in the production and function of urase (Price and Morel 1991) and nitrogenase (Paerl et al. 1993) both are used to sequester nitrogen from the environment. In both studies, the

addition of micronutrients stimulated the production of urase (nickel) and nitrogenase (iron and molybadate) to meet nitrogen demand in nutrient deficient systems.

Micronutrients such as magnesium and zinc are essential for proper function of alkaline phosphatase which leads to the possibility of APA production being limited by these micronutrients. The increase in alkaline phosphatase activity observed in Lake Tanganyika periphyton grown in media with high concentrations of these ions in the absence of phosphate suggests that cultures were exhibiting both phosphorus and micronutrient limitation.

The results of this study show that intact periphyton community enzyme activity is possibly limited by surface area due to boundary layer effects. A boundary layer exists when flow is parallel to the surface of the object creating an area of laminar flow. Dissolved nutrients can only pass through this area by diffusing across the boundary layer (Jones et al. 2000). The effects of boundary layer kinetics were occurring in this study and supports previous studies that demonstrate phosphorous conversion and uptake is predominately a surface process. The majority of phosphorus movement is done within the periphyton polysaccharide matrix in periphyton with high biomass (Steinman et al 1995).

Internal and boundary layer recycling can account for most of the phosphate tumover in intact periphyton than uptake from the water column (Riber and Wetzel 1987). Most common measurements of APA are conducted on disturbed periphyton samples. The decision whether to measure intact or disturbed periphyton lies with the questions you wish to answer. Smucker et al. (2009) sampled intact and disturbed periphyton, finding that disturbed periphyton had greater rates of enzyme activity and

had much greater deviation. Disturbed periphyton sampling would be best suited for total nutrient turnover within the periphyton mat whereas intact periphyton sampling provides the best measure of importance at the ecosystem level where intact periphyton is the natural state. Measuring intact periphyton communities allows for the measurement of phosphorous flux into the periphyton mat.

Conclusion

It has been well established that hard water (alkaline) lakes are more productive, in terms of fish mass, than soft water lakes (Ball 1945, Geagan and Allen 1960). Alkalinity is often associated with saline (high conductivity) lakes as calcium and magnesium contribute both to pH elevation and specific conductance. However, this relationship with conductivity and productivity has been overlooked in recent studies. The results of this study clearly demonstrate that conductivity has important effects on epilithic periphyton in freshwater alkaline lakes and can help explain the high rates of production occurring in nutrient poor systems.

References

Al-Shehri, A.M. 2006. Factors Affecting Alkaline Phosphatase Activity of the Marine Cyanobacterium Lyngbya majuscula. *Journal of Biological Sciences* **6**:931-935

Ball, R.C. 1945. A summary of experiments in Michigan lakes on the elimination of fish populations with rotenone, 1934-1942. *Trans. Am. Fisheries Soc.,* 75: 139-146.

Bootsma H. A. & Hecky, R. E.. 2003. A comparative introduction to the biology and limnology of the African Great Lakes. *Journal of Great Lakes Research* 29:3-18.

Chrost R. J. & Overbeck, J. 1987. Kinetics of Alkaline Phosphatase Activity and Phosphorus Availability for Phytoplankton and Bacterioplankton in Lake Plusssee North German. *Microbial Ecology* 13:229-248.

Currie D. J. & Kalff, J. 1984. A Comparison of the Abilities of Fresh Water Algae and Bacteria to Acquire and Retain Phosphorus. *Limnology and Oceanography* 29:298-310.

Danger, M., Daufresne, T., Lucas, F., Pissard, S., & Lacroix, G. 2008. Does Liebig's law of the minimum scale up from species to communities? *Oikos*, 117, 1741-1751.

Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., & Smith, J. E. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters*, 10: 1135-1142.

Fairchild, G.W., Lowe, R.L., & Richardson, W.B. 1985. Algal periphyton growth on nutrient-diffusing substrates: an in situ bioassay. *Ecology*, 66:465-472.

Hammer, U.T. 1981. Primary production in saline lakes: a review. *Hydrobiologia*, 81:47-58.

Harpole, W. S., Ngai, J. T., Cleland, E. E., Seabloom, E. W., Borer, E. T., Bracken, M. E., Elser, J. J., Gruner, D. S., Hillebrand, H., Shurin, J. B., & Smith, J. E. 2011. Nutrient co-limitation of primary producer communities. *Ecology Letters*, 14: 852–862.

Healey F. P., & Hendzel, L. L. 1979. Fluorometric Measurement of Alkaline Phosphatase Activity in Algae. *Freshwater Biology* 9:429-440.

Hecky, R.E. & Fee, E.J. 1981. Primary production and rates of algal growth in Lake Tanganyika. *Limnology and Oceanography*, 26:532-547.

Hecky, R.E., & Hesslein, R.H. 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. *Journal of the North American Benthological Society,* 14: 631-653.

Hernandez, I., Niell, F.X. & Whitton, B. A. 2002. Phosphatase activity of benthic marine algae. An overview. *Journal of Applied Phycology*. 14:475-487.

Higgins, S.N., Hecky, R. E., & Taylor, W.D. 2001. Epilithic nitrogen fixation in the rocky littoral zones of Lake Malawi, Africa. *Limnology and Oceanography* 46:976-982.

Houlton, B. Z., Wang, Y., Vitousek, P. M., & Field, C. B. 2008. A unifying framework for dinitrogen fixation in the terrestrial biosphere. *Nature,* 454:327.

Howarth R, W., Marino, R., Lane, J., & Cole J.J. 1988. Nitrogen fixation in freshwater estuarine and marine ecosystems 1. rates and importance. *Limnology and Oceanography*, 33:669-687.

Jassby, A. D., Goldman, C. R., & Reuter, J. E. 1995. Long-term change in Lake Tahoe (California-Nevada, U.S.A.) and its relation to atmospheric deposition of algal nutrients. *Archiv fuer Hydrobiologie*, 135:1-21.

Jansson, M., H. Olsson, & Pettersson, K. 1988. Phosphatase Origin Characteristics and Function in Lakes. *Hydrobiologia* 170:157-176.

Jones, J. I., Eaton, J. W., & Hardwick, K. 2000. The influence of periphyton on boundary layer conditions: A pH microelectrode investigation. *Aquatic Botany*, *67*:191-206.

Kathuria, S. & Martiny, A.C. 2011. Prevalence of calcium-based alkaline phosphatase associated with the marine Cyanobacterium Prochlorococcus and other ocean bacteria. *Environmental Microbiology* 13: 74-83.

Kim E. E., & Wyckoff, H. W. 1991. Reaction Mechanism of Alkaline Phosphatase Based on Crystal Structures Two-Metal Ion Catalysis. *Journal of Molecular Biology* 218:449-464.

Kruskopf, M., & Du Plessis, S. 2006. Growth and filament length of the bloom forming Oscillatoria simplicissima (Oscillatoriales, Cyanophyta) in varying N and P concentrations. *Hydrobiologia*, *556:*357-362.

Livingstone, D. & Whitton, B. A. 1984. Water Chemistry and Phosphatase Activity of the Blue-Green Alga *Rivularia* in Upper Teesdale Streams England UK. *Journal of Ecology* 72:405-422.

Loeb, S.L. 1981. An in-situ method for measuring the primary productivity and standing crop of the epilithic periphyton community in lentic systems. *Limnology and Oceanography*, *26*: 394-399.

O'Reilly, C. M. 2006. Seasonal dynamics of periphyton in a large tropical lake. *Hydrobiologia*, *553*:293-301.

Paerl, H. W., Joye, S. B., & Fitzpatrick, M. 1993. Evaluation of nutrient limitation of CO-2 and N-2 fixation in marine microbial mats. *Marine Ecology Progress Series*,

101:297-306.

Price, N.M., & Morel, F.M. 1991. Colimitation of phytoplankton growth by nickel and nitrogen. *Limnology and Oceanography*, 36:1071-1077.

Reeders, H.H., Boers, P.C.M., Van Der Molen, D.T., & Helmerhorst, T.H. 1998. Cyanobacterial dominance in the lakes Veluwemeer and Wolderwijd, The Netherlands. *Water Science and Technology*, 37:85-92.

Riber, H.H. & Wetzel, R.G. 1987. Boundary-layer and internal diffusion effects on phosphorus fluxes in lake periphyton. *Limnology and Oceanography*, *32*:1181-1194.

Rose, C. & Axler, R.P. 1997. Uses of alkaline phosphatase activity in evaluating phytoplankton community phosphorus deficiency. *Hydrobiologia*, *361*:145-156.

Smucker, N. J., DeForest, J. L., & Vis, M. L. 2009. Different methods and storage duration affect measurements of epilithic extracellular enzyme activities in lotic biofilms. *Hydrobiologia*, 636:153-162.

Steinman, A.D. & Mcintire, C.D. 1987. Effects of irradiance on the community structure and biomass of algal assemblages in laboratory streams. *Canadian Journal of Fisheries and Aquatic Sciences*, 44:1640-1648.

Steinman, A.D., Mulholland, P.J., & Beauchamp, J.J.. 1995. Phosphorus cycling and biomass accrual in stream periphyton communities. *Journal of the North American Benthological Society* 14: 371-381.

Treseder, K.K. & Vitousek, P.M. 2001. Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests. *Ecology* 82:946-954.

Vadeboncoeur Y., Lodge, D. M., & Carpenter, S.R. 2001. Whole-lake fertilization effects

on distribution of primary production between benthic and pelagic habitats. *Ecology. 82:1065*-1077.

Vadeboncoeur Y., Peterson, G.M., Zanden, J., & Kalff, J. 2008. Benthic algal production across lake size gradients: Interactions among morphometry, nutrients, and light. *Ecology*. 89:2542-2552.

Vadeboncoeur Y., Jeppesen, E., Vander Zanden, M. J., Schierup, H., Christoffersen, K., & Lodge, D.M. 2003. From Greenland to green lakes: Cultural eutrophication and the loss of benthic pathways in lakes. *Limnology and Oceanography* 48:1408-1418.

Vander Zanden M. J., & Vadeboncoeur, Y. 2002. Fishes as integrators of benthic and pelagic food webs in lakes. *Ecology*. 83:2152-2161.

Vander Zanden M. J., Chandra, S., Park, S., Vadeboncoeur, Y., & Goldman, C. R. 2006. Efficiencies of benthic and pelagic trophic pathways in a subalpine lake. *Canadian Journal of Fisheries and Aquatic Sciences.* 63:2608-2620.

Wetzel, R.G. 2001. Limnology: lake and river ecosystems. San Diego, CA: Academic Press.

Table 1. Polyculture media recipe used in the 2010 and 2011 laboratory experiments.

$MgSO_4$	14.29 g
$CaCl_2$	6.61 g
Na_2CO_3	7.43 g
$Na_2SiO_3*5H_2O$	3.55 g
Citric Acid	2.23 g
$FeSO_4$	1.86 g
Vit. B12	.025 g
$Co(NO_3)_2$	0.5 mL
H_3BO_3	0.025 g
Trace Element	0.5 mL

Table 2. Analysis of Variance results comparing area-specific alkaline phosphatase activity of Lake Tahoe periphyton between conductivity and phosphorus treatments in the 2010 experiment. Significant results indicated by an asterisk.

	Df	Sum Sq	Mean Sq	F Value	p
Conductivity	1	0.00308	0.00308	0.4463	0.5136
Phosphorus Source	1	0.59722	0.59722	86.6244	<0.0001*
Conductivity x Phosphorus Source	1	0.00014	0.00014	0.021	0.8867
Residuals	16	0.11031	0.00689		

Table 3. Analysis of Variance results comparing area-specific alkaline phosphatase activity of Lake Tahoe periphyton between conductivity and phosphorus treatments in the 2011 experiment. Significant results indicated by an asterisk.

	Df	Sum Sq	Mean Sq	F Value	p
Conductivity	1	0.0043	0.0043	0.2005	0.6602972
Phosphorus Source	1	3.3746	3.3746	155.656	<0.0001*
Conductivity x Phosphorus Source	1	0.4809	0.4809	22.1818	0.0005*
Residuals	16	0.3469	0.0217		

Table 4. Analysis of Variance results comparing area-specific alkaline phosphatase activity of Lake Tanganyika periphyton between conductivity and phosphorus treatments in the 2010 experiment. Significant results indicated by an asterisk.

	Df	Sum Sq	Mean Sq	F Value	p
Conductivity	1	1.14571	1.14571	27.246	<0.0001*
Phosphorus Source	1	2.05721	2.05721	48.922	<0.0001*
Conductivity x Phosphorus Source	1	0.78786	0.78786	18.736	0.0005*
Residuals	16	0.67281	0.04205		

Table 5. Analysis of Variance results comparing area-specific alkaline phosphatase activity of Lake Tanganyika periphyton between conductivity and phosphorus treatments in the 2011 experiment. Significant results indicated by an asterisk.

	Df	Sum Sq	Mean Sq	F Value	p
Conductivity	1	1.5305	1.5305	16.443	0.001*
Phosphorus Source	1	6.0065	6.0065	64.5308	<0.0001*
Conductivity x Phosphorus Source	1	0.0113	0.0113	0.1216	0.7318652
Residuals	16	1.4893	0.0931		

Table 6. Analysis of Variance results comparing periphyton dry weight of Lake Tahoe periphyton between conductivity and phosphorus treatments in the 2010 experiment. Significant results indicated by an asterisk.

	Df	Sum Sq	Mean Sq	F Value	p
Conductivity	1	19098.7	19098.7	63.9694	<0.0001*
Phosphorus Source	1	630.8	630.8	2.1128	0.1654
Conductivity x Phosphorus Source	1	66.3	66.3	0.221	0.6438
Residuals	16	4777	298.6		

Table 7. Analysis of Variance results comparing periphyton dry weight of Lake Tahoe periphyton between conductivity and phosphorus treatments in the 2011 experiment. Significant results indicated by an asterisk.

	Df	Sum Sq	Mean Sq	F Value	p
Conductivity	1	2093.06	2093.06	18.4347	<0.0001*
Phosphorus Source	1	156.8	156.8	1.381	0.25712
Conductivity x Phosphorus Source	1	279.75	279.75	2.4639	0.136051
Residuals	16	1816.63	113.54		

Table 8. Analysis of Variance results comparing periphyton dry weight of Lake Tanganyika periphyton between conductivity and phosphorus treatments in the 2010 experiment. Significant results indicated by an asterisk.

	Df	Sum Sq	Mean Sq	F Value	p
Conductivity	1	23505.1	23505.1	149.0517	<0.0001*
Phosphorus Source	1	0.1	0.1	0.0006	0.9807
Conductivity x Phosphorus Source	1	2334.5	2334.5	14.8038	0.001423*
Residuals	16	2523.2	157.7		

Table 9. Analysis of Variance results comparing periphyton dry weight of Lake Tanganyika periphyton between conductivity and phosphorus treatments in the 2011 experiment. Significant results indicated by an asterisk.

	Df	Sum Sq	Mean Sq	F Value	p
Conductivity	1	*65*	3765	20.221	<0.0001*
Phosphorus Source	1	5.4	5.4	0.029	0.866939
Conductivity and Phosphorus Source	1	140.4	140.4	0.754	0.39804
Residuals	16	2979.1	186.2		

Table 10. Analysis of Variance results comparing biomass-specific alkaline phosphatase activity of Lake Tahoe periphyton between conductivity and phosphorus treatments in the 2010 experiment. Significant results indicated by an asterisk.

	Df	Sum Sq	Mean Sq	F Value	p
Conductivity	1	2.3610e-06	2.3610e-06	2.5261	0.1315
Phosphorus Source	1	3.2257e-05	3.2257e-05	34.5112	<0.0001*
Conductivity x Phosphorus Source	1	2.3610e-06	2.3610e-06	2.5261	0.1315
Residuals	16	1.4955e-05	9.3500e-07		

Table 11. Analysis of Variance results comparing biomass-specific alkaline phosphatase activity of Lake Tahoe periphyton between conductivity and phosphorus treatments in the 2011 experiment. Significant results indicated by an asterisk.

	Df	Sum Sq	Mean Sq	F Value	p
Conductivity	1	3.2315e-06	3.2315e-06	5.0712	<0.05*
Phosphorus Source	1	2.2049e-05	2.2049e-05	34.6011	<0.0001*
Conductivity x Phosphorus Source	1	3.2315e-06	3.2315e-06	5.0712	<0.05*
Residuals	16	1.0196e-05	6.3720e-07		

Table 12. Analysis of Variance results comparing biomass-specific alkaline phosphatase activity of Lake Tanganyika periphyton between conductivity and phosphorus treatments in the 2010 experiment. Significant results indicated by an asterisk.

	Df	Sum Sq	Mean Sq	F Value	p
Conductivity	1	0.00024492	0.00024492	10.497	<0.0001*
Phosphorus Source	1	0.00043310	0.00043310	18.562	<0.0001*
Conductivity x Phosphorus Source	1	0.00024492	0.00024492	10.497	<0.0001*
Residuals	16	0.00037331	0.00002333		

Table 13. Analysis of Variance results comparing biomass-specific alkaline phosphatase activity of Lake Tanganyika periphyton between conductivity and phosphorus treatments in the 2011 experiment. Significant results indicated by an asterisk.

	Df	Sum Sq	Mean Sq	F Value	p
Conductivity	1	0.00010468	1.0468e-04	9.1843	<0.001*
Phosphorus Source	1	0.00021974	2.1974e-04	19.2794	<0.0001*
Conductivity x Phosphorus Source	1	0.00010468	1.0468e-04	9.1843	<0.001*
Residuals	16	0.00018236	1.1398e-05		

Figure 1. Area-specific alkaline phosphatase activity from the 2010 and 2011 conductivity experiment for each of the four treatments by lake. Lake Tahoe is represented by black bars and grey bars represent Lake Tanganyika periphyton. Error bars represent standard error. Treatments are represented as high conductivity plus organic phosphorous (HO), low conductivity plus organic phosphorus (LO), high conductivity plus inorganic phosphorus (HI), and low conductivity plus inorganic phosphorus (LI). Lake Tanganyika had significantly higher rates of APA compared to Lake Tahoe in both years (p-value = 0.05). Lake Tanganyika periphyton in the high conductivity plus organic phosphorous treatment was significantly greater than the low conductivity plus organic phosphorous treatment in 2010 and 2011 (p-value = 0.0001 and 0.001).

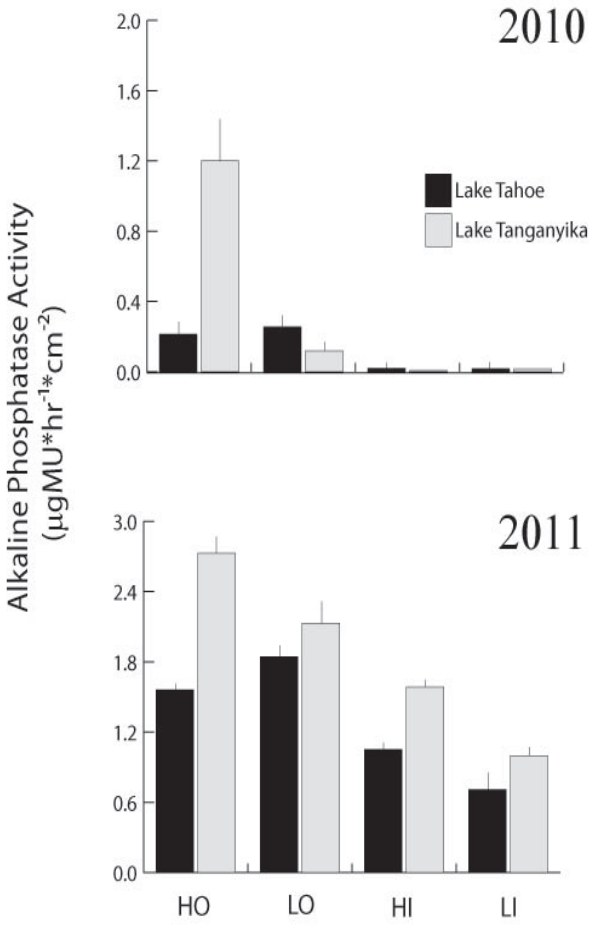


Figure 2. Periphyton dry mass from the 2010 and 2011 conductivity experiment for each of the four treatments by lake. Lake Tahoe is represented by black bars and grey bars represent Lake Tanganyika periphyton. Error bars represent standard error. In both 2010 and 2011, the high conductivity treatments plus inorganic or organic phosphorous had significantly greater periphyton biomass compared to the low conductivity treatments in cultures from both lakes (p-value = <0.0001).

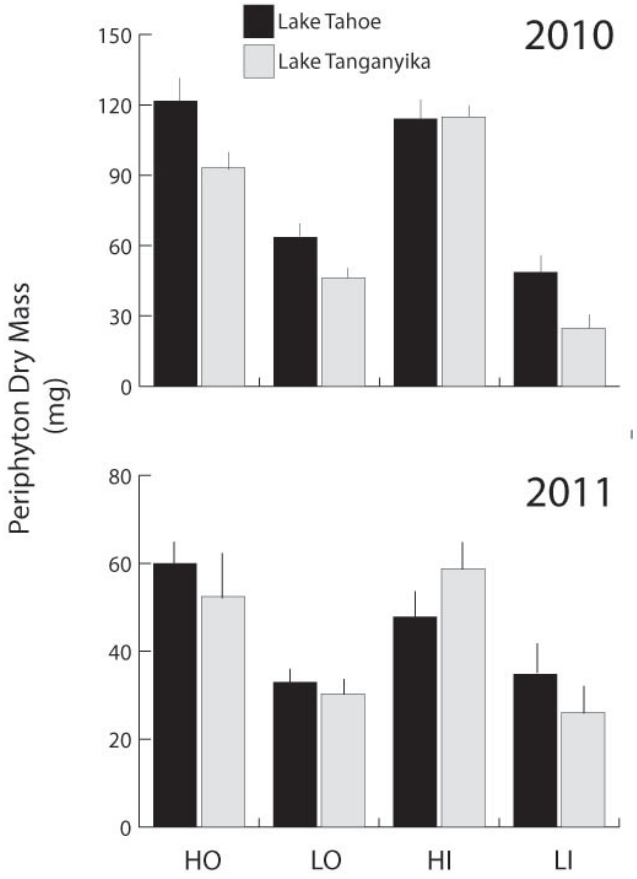


Figure 3. Periphyton area-specific gross primary productivity from the 2010 and 2011 conductivity experiment for each of the four treatments by lake. Lake Tahoe is represented by black bars and grey bars represent Lake Tanganyika periphyton. Error bars represent standard error. In both 2010 and 2011, the high conductivity treatments plus inorganic or organic phosphorous had significantly greater periphyton biomass compared to the low conductivity treatments in cultures from both lakes (p-value = <0.0001).

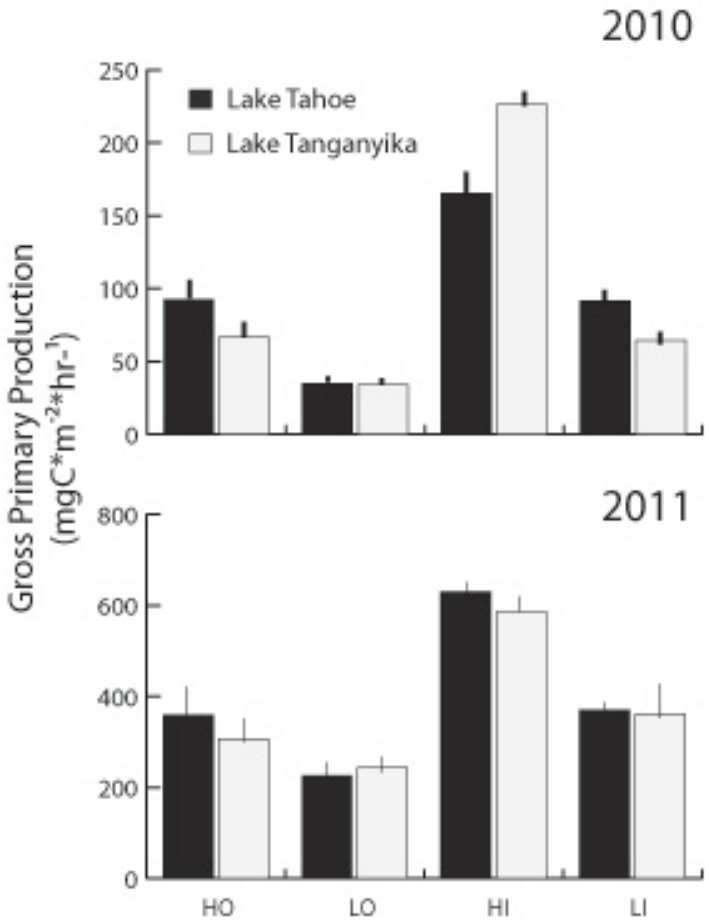


Figure 4. Alkaline phosphatase activity by periphyton dry mass relationship. Lake Tahoe is represented by black bars and grey bars represent Lake Tanganyika periphyton. Treatments are represented as high conductivity plus organic phosphorous (x), low conductivity plus organic phosphorus (-), high conductivity plus inorganic phosphorus (o), and low conductivity plus inorganic phosphorus (+). No significant relationship was observed between periphyton biomass and periphyton alkaline phosphatase.

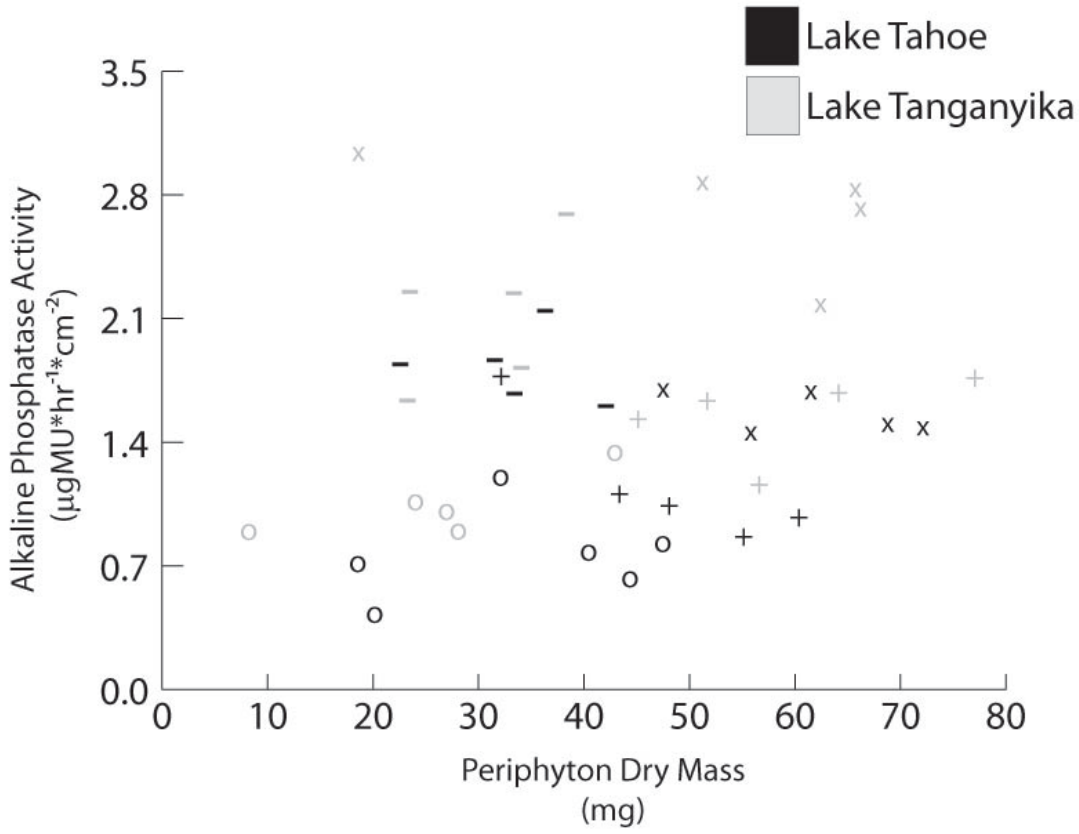


Figure 5. Nitrogen fixation rates by periphyton dry mass relationship. Lake Tahoe is represented by black bars and grey bars represent Lake Tanganyika periphyton. Treatments are represented as high conductivity plus organic phosphorous (x), low conductivity plus organic phosphorus (-), high conductivity plus inorganic phosphorus (o), and low conductivity plus inorganic phosphorus (+). No significant relationship was observed between periphyton biomass and periphyton alkaline phosphatase.

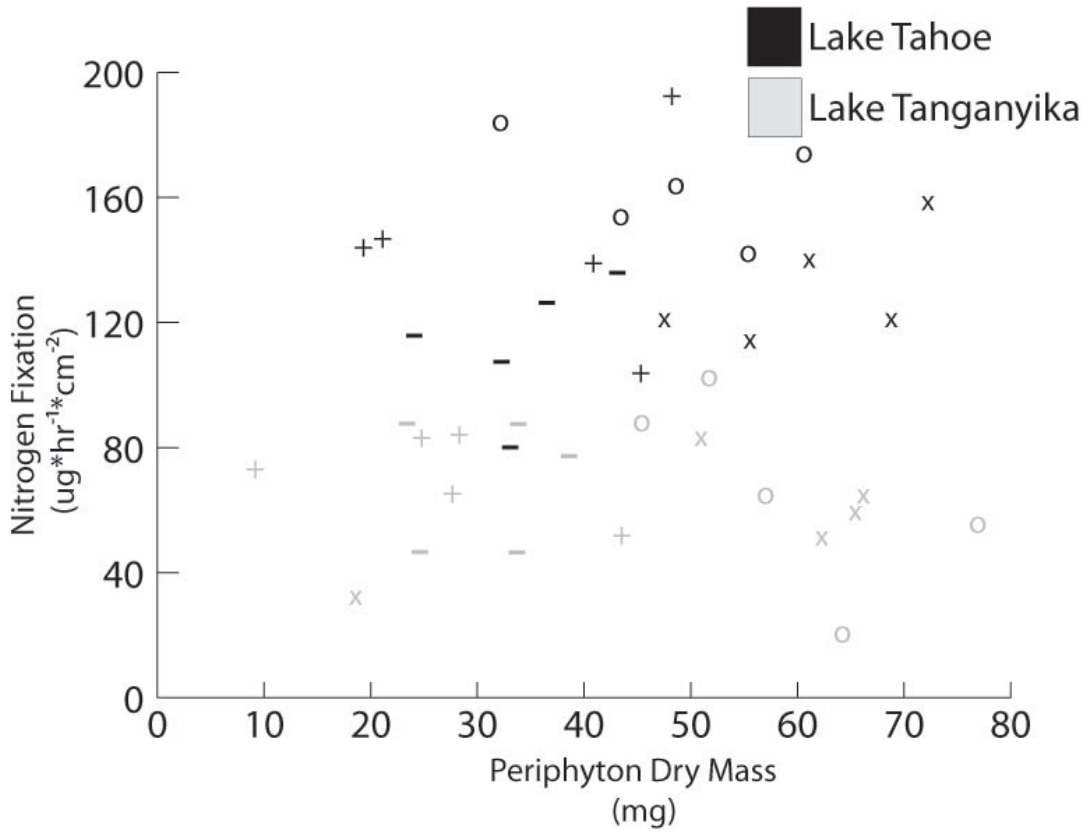


Figure 6. Biomass-specific alkaline phosphatase activity from the 2010 and 2011 conductivity experiment for each of the four treatments by lake. Lake Tahoe is represented by black bars and grey bars represent Lake Tanganyika periphyton. Error bars represent standard error. In both years, biomass-specific alkaline phosphatase activity was significantly higher than the inorganic phosphorous treatments for both lakes (p-value= 0.0001).

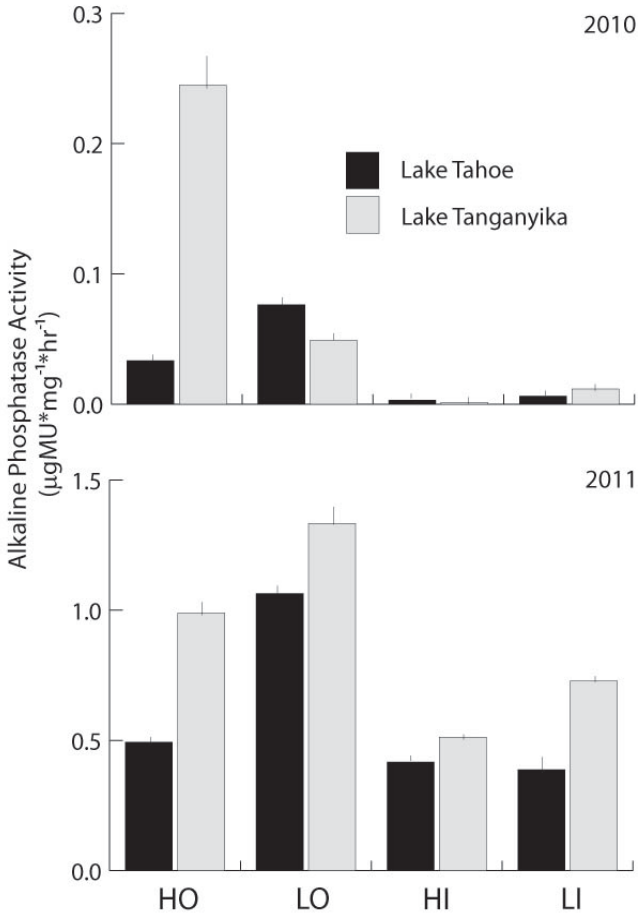
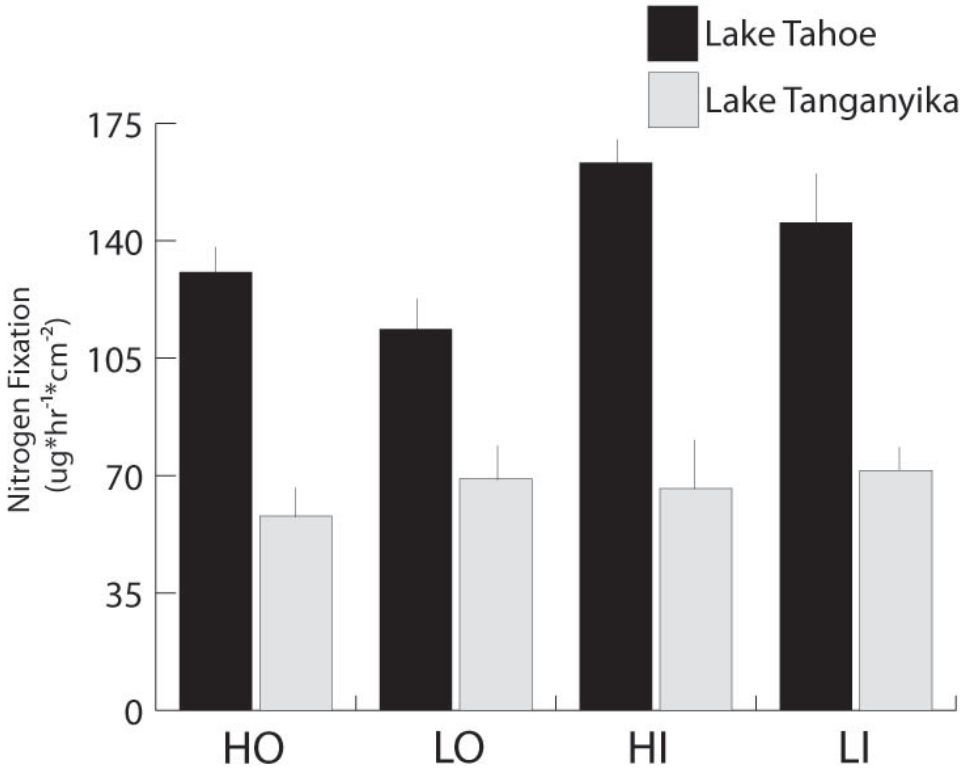


Figure 7. Nitrogen fixation rates from the 2011 conductivity experiment for each of the four treatments by lake. Lake Tahoe is represented by black bars and grey bars represent Lake Tanganyika periphyton. Error bars represent standard error. Cultures from Lake Tahoe had significantly higher rates of nitrogen fixation compared to Lake Tanganyika periphyton cultures (0.05).



Introduction

Despite low nutrient availability, Lake Tanganyika is one of the most productive tropical lakes ever surveyed (Hecky and Fee 1981) supporting a large pelagic fishery producing between 165,000 and 200,000 metric tons annually (Molsa et al. 1999). The lake is a hotspot of diversity with approximately 300 species of fish, 86% of those occurring in the littoral zone (Vadeboncoeur et al. 2011). Although the littoral zone is small relative to the pelagic zone in terms of surface area, the majority of species, especially cichlids, are concentrated in the shallow water near the shore (littoral) zone (Coulter 1991).

Periphyton primary production in Lake Tanganyika is incredibly efficient, having high rates of carbon fixation with low periphyton biomass in a very nutrient poor environoment (O'Reilly 2006). High productivity in this nutrient poor system suggests rapid nutrient recycling is occurring in the littoral zone. Nutrient recycling within a system releases nutrients that are sequestered in primary producer biomass back into the nutrient pool of the system through biological processes (digestion and decomposition). Grazing, particularly by fish, can indirectly stimulate productivity through excretion and defecation of inorganic nutrients (McCollum et al. 1998). Fish excretion and defecation can supply 48% of the inorganic nitrogen and 46% of the inorganic phosphorus demand of littoral periphyton in Lake Tanganyika (Emidio et al. 2003). Nutrients (nitrogen and phosphorous) released through grazing in the littoral zone, coupled with high light availability, results in increased primary production efficiency in the littoral zone. Fish, and other organisms, of different trophic levels throughout the food web are dependent on the carbon fixed by littoral benthic primary

production in oligotrophic systems (Hecky and Hesslein 1995, Vander Zanden and Vadeboncoeur 2002).

In addition to the recycling of nutrients by grazing fish, the periphyton community structure is strongly influenced by grazing. Diatoms, superior competitors for nutrients in the periphyton community, can quickly overrun the other members of the periphyton community in the absence of grazers in oligotrophic systems (Power et al. 1988). Community structure of the periphyton will shift with diatoms being the dominant periphyton component. This change in community composition reduces the abundance of cyanobacteria which can supply the littoral ecosystem with additional sources of nitrogen and phosphorous through enzymatic processes. Some cyanobacteria species produce high levels of extracellular enzymes (alkaline phosphatase and nitrogenase) used to convert bio-unavailable nutrients to a more useable form (Healey and Hendzel 1979, Currie and Kalff 1984, Livingstone and Whitton 1984). By decreasing cyanobacteria abundance in the periphyton community, sources of nitrogen and phosphorus from cyanobacteria enzyme activity can also be reduced. The intent of this study being is to identify fish grazing affects the ability of periphyton to sequester phosphorus through alkaline phosphatase activity.

As discussed in chapter one, alkaline phosphatase is an enzyme produced by many organisms. Phosphatase converts organic phosphorus compounds into useable orthophosphate compounds (Jansson et al. 1988). The efficiency of alkaline phosphatase activity is strongly regulated by the presence of inorganic phosphorous, and thus, APA rates are highest when inorganic phosphate is limiting (Jansson et al. 1988, Chrost and Overbeck 1987, Hernandez et al. 2002). Phosphorus release through

nutrient recycling by organisms such as fish could inhibit the production of alkaline phosphatase activity in periphyton if fish biomass is high. Kalhert and Petterson (2002) demonstrated inhibition of alkaline phosphates activity of periphyton growing on living heterotrophs compared to periphyton on non-living and living autotrophic substrata. The results of this study indicate that nutrients released by excretion are an essential component of periphyton nutrient requirements. In conditions of phosphorous scarcity, alkaline phosphatase activity is expected to be high.

Lake Tanganyika is a large oligotrophic lake with remarkably low concentrations of both inorganic (~0.1µmol*L^{-1}) and organic (~0.04 µmol*L^{-1}) phosphorous (Corman et al 2010). The periphyton community in Lake Tanganyika is dominated by the heterocystous cyanobacteria genera *Calothrix*. *Calothrix* is an eyelash shaped filamentous genus of Cyanobacteria that grows attached to surfaces with a basal heterocyst (Wehr and Sheath 2003). Basal regeneration provides Calothrix a mechanism to cope with grazing without decreases in nitrogen fixation. If the top portion of the individual is removed, the biomass lost will be regenerated from the basal component anchored to the substratum. Periphyton community composition is shaped by grazing, with grazing favoring taxa with basal cells and regenerations (Rosemond et al. 2000). Grazing fish reduce diatom biovolume through selective grazing resulting in an increase in cyanobacterial biovolume (McCollum et al. 1998). In areas experiencing high grazing pressure, such as the littoral zone of Lake Tanganyika, diatom biomass will be suppressed. Suppression of diatom competitors allows cyanobacteria to persist in the ecosystem (Power et al 1988).

Curiously, nitrogen fixation is related to phosphorous availability (Sañudo-Wilhelmy et al. 2001) while phosphatase production is nitrogen intense (Treseder and Vitousek 2001) creating a nutrient paradox in oligotrophic littoral systems. Production of phosphatase enzymes would be expected to decrease in the presence of grazing fish because of the inorganic phosphorous released through excretion. However, phosphorous excreted by grazing fish is inversely related to fish biomass (Vanni 2002) implying that fish act as sinks of phosphorous (Allan and Castillo 2007), ultimately reducing phosphorous available for periphyton in the littoral zone.

Both nutrient recycling and community structure alterations through fish grazing have the potential to alter periphyton available nutrients in oligotrophic lakes. The purpose of this study is to identify the relationship between fish grazing and phosphorus acquisition. I hypothesize that in the presence of fish, periphyton alkaline phosphatase activity will be suppressed due to the increase in available inorganic phosphorous in relation to fish biomass.

Methods

I conducted several experiments, both in the field and laboratory, to better understand how grazing pressure affects alkaline phosphatase activity in natural systems. Additionally, I investigated potential differences in alkaline phosphatase activity in space within Lake Tanganyika.

Fish Grazing

2010 Field Study: To test the effects of fish grazing on alkaline phosphatase activity, I conducted an experiment on littoral periphyton in Lake Tanganyika, Tanzania (June through August 2010). I selected three sites within the lake based on littoral fish

density and periphyton primary production. Previous experiments have shown that primary production is positively, but weakly, correlated to fish density. Based on this, I selected three sites, one high fish density and primary production and two sites with moderate fish density and periphyton primary production.

I conducted the experiments on large boulders at 5 meters depth. At each site of the three sites, there were two treatments (ungrazed and control), with three replicates each. I manipulated fish grazing by using 900cm^2 exclosures (5mm mesh). Measurements of alkaline phosphatase activity were taken (time 0) and then the exclosures were anchored to the substratum using cobbles. I allowed algae in the exclosures to grow undisturbed for 5 weeks prior to the final sampling.

I measured alkaline phosphatase activity (APA) as the production of fluorescent 4-Methylumbelliferyl (4-MU) from non-fluorescent 4-Methylumbellifery phosphate (4-MUP) substrate. Assays were done in adjustable volume, bottomless incubation chambers (~1000mL total volume) anchored by weights. Twenty milliliters of liquid 4-MUP (300µM) substrate (prepared daily) was injected into each chamber (3 exclosures, 3 grazed, and one blank) and allowed to incubate for forty minutes at depth. The chambers were then stirred, using stir bar mounted in the lid of the incubation chambers, and twenty milliliters of sample were collected from each chamber using a 20mL syringe. Fluorescence was analyzed immediately using a QuantiFluor hand held fluorometer (Promega Corporation, Madison Wisconsin) equipped with an ultraviolet lamp. I calibrated the QuantiFluor fluorometer weekly using 25ugMU/L stock solution to ensure accuracy. Alkaline phosphatase activity was corrected for incubation time, surface area, assay volume, and natural fluorescence of the substrate. Among site

variation in alkaline phosphatase was done following the same procedure at all 12 field sites.

2011 Laboratory Study: Fish grazing experiments were conducted from January to February 2011. The experiment was a regression design to test nutrient recycling and periphyton response to grazing fish biomass. Six 150L aquaria were lined with 40 unglazed clay tiles, inoculated with cultures collected from Lake Tanganyika, and allowed to colonize in the presence of grazing by *Tropheus duboisi*. After colonization, the tanks were then divided with each tank having a grazed side and a side protected from grazing. Each tank randomly received a fish density (by mass) of 0.00, 2.70, 7.60, 9.40, 16.80, or 33.50g.

APA: Tiles were placed into sterile Whirl-Pak sample bags with 100mL of water from the tanks, enough to completely cover the tiles. For each tank and each treatment, 100mL of water from the aquarium was used as a blank. Five milliliters of liquid 4-MUP (300µM) substrate was added to each sample and blank, incubated for 40 minutes under direct light. After incubation, three 5mL aliquots were collected from each bag and transferred to glass cuvettes for analysis. Alkaline phosphatase activity (APA) was measured using the fluorescent method as described above.

33P Uptake: Tiles were placed into 16oz. Nalgene plastic wide-mouthed jars with 300mL filtered tank water. To each chamber, 0.5mL of 0.1uCi of carrier-free phosphate was added and the samples were mixed using a shaker table set at 60rpm. Four replicates, including one blank, per tank were run. Samples were taken from each chamber at times 0, 5, 10, 15, 30, 45, 60, and 120min. Samples were pipetted into 8mL plastic scintillation vials for analysis. Samples were analyzed using a Beckman Coulter

LS6500 multipurpose scintillation counter. Samples were counted for two minutes and recorded as disintegrations per minute (DPM). Phosphorus uptake was determined as the loss of phosphorus radiotracer from the water column. Blanks from each tank for each replicate were run to account for the natural loss of radiotracer from the water column not incorporated into periphyton. Rates of phosphorus uptake were calculated by subtracting final DPM from initial DPM correcting for incubation time and surface area.

Statistical Analysis: I preformed all statistical analyses using the "R: A Language and Environment for Statistical Computing" software (R Development Core Team, Vienna, Austria). I conducted analysis of variance to determine the effects of fish grazing on alkaline phosphatase activity and phosphate uptake for field (2010) and laboratory (2011) fish grazing experiments. I log transformed data to meet the assumptions of the ANOVA model. Assumptions were tested using the Bartlett's Test of Homogeneity of Variances (p-value > 0.05) and the Shapiro-Wilk Normality Test (p-value > 0.05).

Results

Fish grazing: Despite dramatic differences in periphyton biomass, grazing pressure had little impact on area-specific periphyton alkaline phosphatase activity in both the laboratory and field studies. In the 2010 field study, there were no significant differences between periphyton APA in the experimental and control treatment at the three sites (Figure 1). Similar results were observed in the 2011 laboratory study. There was no significant difference in area-specific periphyton alkaline phosphatase activity (Figure 2) and phosphorous (33P) uptake (Figure 3) between the experimental

and control treatment. Little effect of fish density on area-specific APA was observed. However, periphyton grown in aquarium 1 had significantly less area-specific APA compared to the other 5 aquaria. The fact that aquarium 1 had lower rates of APA but no difference in 33P uptake, in addition to production data, suggests an error in the experimental execution. We speculate that aquarium 1 had phosphorous contamination before the experiment began which affected the results of the experiment. The results from the fish grazing experiments (laboratory and field) suggest that periphyton biomass removal does not negatively affect organic phosphorous conversion (APA) or phosphorous acquisition (33P) by periphyton.

Among Site Variability: The 12 site survey conducted in the summer of 2010 demonstrated the stability of area-specific alkaline phosphatase activity in this system. Across the 12 sites surveyed, there was no significant difference among individual sites or between the same site on different sampling dates (Figure 4). This suggests that there is no significant change in area-specific APA between sites and between different sampling dates.

Discussion

The results of this study do not support the hypothesis that periphyton alkaline phosphatase activity will be suppressed in the presence of grazing fish, through inorganic phosphorous excretion. The results of the fish grazing experiments showed similar trends in both the laboratory and field experiments. There appears to be no difference in alkaline phosphatase activity or phosphorous uptake between experimental and control treatments despite the dramatic difference in periphyton biomass. The data gathered in these sets of experiments also show much lower

alkaline phosphatase activity than previously published reports. There are two possible explanations for the observed data. Total levels of organic phosphorous in the field and laboratory experiments could have been low reducing enzyme activity through substrate (organic phosphorous) limitation. Previous work has demonstrated that the water column of Lake Tanganyika has incredibly low levels of organic phosphorous (Corman et al 2010). Another possible explanation, as in chapter one, is that interactions between the periphyton community and the nutrients in the water column primarily limited by surface area.

Many studies have related grazing pressures to periphyton performance. Grazing has negative effects on periphyton biomass, chlorophyll a concentration, and area specific primary production (Rosemond 1993). Rosemond (1993) identified periphyton function as being controlled by grazing snails, in addition to illumination and nutrient levels, in small order streams. These studies illustrate the effects of grazers on periphyton communities, however little work has been done on fish grazing and its effects on the periphyton and few studies have attempted to quantify periphyton alkaline phosphatase activity response to herbivory.

This lack of a response to biomass removal suggests boundary layer effects are affecting phosphorous conversion and uptake of the periphyton in this study. A boundary layer exists when flow is parallel to the surface of the object creating an area of laminar flow. Dissolved nutrients can only pass through this area by diffusing across the boundary layer (Jones et al. 2000). This restriction of periphyton and water column interaction, through boundary layer effects, has ramifications on periphyton metabolism (Riber and Wetzel 1987).

Fertilization experiments have demonstrated that nutrient uptake from the water column is mainly a surface process. A study by Horner et al. (1990) showed that although phosphorous uptake was stimulated by nutrient additions but uptake rate was limited by periphyton thickness. The effects of boundary layer kinetics was evident in this study and supports previous studies that demonstrate phosphorous conversion and uptake is predominately a surface process and the bulk of phosphorus movement is done within the periphyton polysaccharide matrix.

Internal and boundary layer recycling can account for most of the phosphate tumover in intact periphyton than uptake from the water column (Riber and Wetzel 1987). Typically, alkaline phosphatase activity is measured using a disrupted periphyton mat corrected for chlorophyll a concentration, a proxy for periphyton biomass, and not surface area. By disrupting the periphyton mat, boundary layer effects are removed and accurate measures of alkaline phosphatase contributions to ecosystem processes cannot be made. Measurements on intact periphyton provide the best measure of importance of alkaline phosphatase activity at the ecosystem level.

Conclusions

Alkaline phosphatase activity measured in the field and laboratory studies were lower than other published reports (Kahlert and Petterson 2002). There are two possible explanations for this, the first being the difference in sampling methods. The majority of studies measuring alkaline phosphatase activity use disturbed periphyton instead of intact (as used in this study) removing boundary layer kinetics on periphyton and water column nutrient exchange. Another possible explanation for the results

observed from this set of experiments is the low levels of organic phosphorous in the environment. Most likely, both possible explanations are plausible in this system (laboratory and field).

The high rates of periphyton primary production observed in the littoral zone of Lake Tanganyika, despite low periphyton biomass, could be attributed to efficient nutrient recycling mechanisms in Lake Tanganyika (O'Reilly 2006). Although alkaline phosphatase activity is a possible source of inorganic phosphorous in a nutrient poor system, APA is not responsible for the high rates of primary production observed in Lake Tanganyika. Fish excretion is most likely the driving force behind the high rates of primary production observed in the littoral zone of Lake Tanganyika.

References

Allan, J.D. & Castillo, M.M. 2007. Stream Ecology: structure and function of running waters. New York City, NY: Springer Publishing.

Chrost R. J. & Overbeck, J. 1987. Kinetics of Alkaline Phosphatase Activity and Phosphorus Availability for Phytoplankton and Bacterioplankton in Lake Plusssee North Germany. *Microbial Ecology* 13:229-248.

Corman, J. R., McIntyre, P. B., Kuboja, B., Mbemba, W., Fink, D., Wheeler, C. W., & Flecker, A. S. 2010. Upwelling couples chemical and biological dynamics across the littoral and pelagic zones of Lake Tanganyika, East Africa. *Limnology and Oceanography*, 55:214-224.

Coulter, G.W. 1991. Lake Tanganyika and its Life. Cary, NC: Oxford University Press.

Currie D. J. & Kalff, J. 1984. A Comparison of the Abilities of Fresh Water Algae and Bacteria to Acquire and Retain Phosphorus. *Limnology and Oceanography* 29:298-310.

Andre, E.R., Hecky, R.E., & Duthie, H.C. 2003. Nitrogen and Phosphorus Regeneration by Cichlids in the Littoral Zone of Lake Malawi, Africa. *Journal of Great Lakes Research*. 29:190-201.

Healey, F. P. & Hendzel, L.L. 1979. Fluorometric Measurement of Alkaline Phosphatase Activity in Algae. *Freshwater Biology*. 9:429-440.

Hecky, R.E. and Fee, E.J. 1981. Primary production and rates of algal growth in Lake Tanganyika. *Limnology and Oceanography*, 26: 532-547.

Hecky, R.E. & Hesslein, R.H. 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. *Journal of the North American Benthological Society,* 14: 631-653.

Hernandez I., Niell, F.X., & Whitton, B.A. 2002. Phosphatase activity of benthic marine algae. An overview. *Journal of Applied Phycology*, 14:475-487.

Horner, R.R., Welch, E,B., Seeley, M.R., & Jacoby, J.M. 1990. Responses of periphyton to changes in current velocity suspended sediment and phosphorus concentration. *Freshwater Biology*, *24*:215-232.

Jansson, M., Olsson, H., & Pettersson, K. 1988. Phosphatase Origin Characteristics and Function in Lakes. *Hydrobiologia,* 170:157-176.

Jones, J. I., Eaton, J.W., & Hardwick, K. 2000. The influence of periphyton on boundary layer conditions: A pH microelectrode investigation. *Aquatic Botany*, 67:191-206.

Kahlert, M. & Pettersson, K. 2002. The impact of substrate and lake trophy on the biomass and nutrient status of benthic algae. *Hydrobiologia*, 489:161-169.

Livingstone, D. & Whitton, B.A. 1984. Water Chemistry and Phosphatase Activity of the Blue-Green Alga *Rivularia* in Upper Teesdale Streams England UK. *Journal of Ecology,* 72:405-422.

McCollum, E.W., Crowder, L.B., & McCollum, A.S. 1998. Complex interactions of fish, snails, and littoral zone periphyton. *Ecology*, 79:1980-1994.

Molsa, H., Reynolds, J.E., Coenen, E.J., & Lindqvist, O.V. 1999. Fisheries research towards resource management on Lake Tanganyika. *Hydrobiologia*,

O'Reilly, C.M. 2006. Seasonal dynamics of periphyton in a large tropical lake. *Hydrobiologia*, 553:293-301

Power, M.E., Stewart, A.J., & Matthews, W.J. 1988. Grazer Control of Algae in an Ozark Mountain Stream: Effects of Short-Term Exclusion. *Ecology*, 69:1894-1898.

Riber, H.H. & Wetzel, R.G. 1987. Boundary-layer and internal diffusion effects on phosphorus fluxes in lake periphyton. *Limnology and Oceanography*, 32:1181-1194.

Rosemond, A.D. 1993. Interactions among irradiance, nutrients, and herbivores constrain a stream algal community. *Oecologia, 94*:585-594.

Rosemond, A.D., Pringle, C.M., Ramirez, A., Paul, M.J., & Meyer, J.L. 2002. Landscape variation in phosphorus concentration and effects on detritus-based tropical streams. *Limnology and Oceanography*, 47:278-289.

Sanudo-Wilhelmy, S.A., Kustka, A.B., Gobler, C. J., Hutchins, D.A., Yang, M., Lwiza, K., & Carpenter, E. J. 2001. Phosphorus limitation of nitrogen fixation by Trichodesmium in the central Atlantic ocean. *Nature*, 411

Treseder, K.K. and Vitousek, P.M. 2001. Effects of soil nutrient availability on investment in acquisition of N and P in Hawaiian rain forests. *Ecology,* 82:946-954.

Vadeboncoeur, Y. & Steinman, A.D. 2002. Periphyton function in lake ecosystems. *The Scientific World Journal*, *2*:1-20.

Vadeboncoeur, Y., McIntyre, P.B., & Vander Zanden, M.J. 2011. Borders of biodiversity: life at the edge of the world's large lakes. *BioScience* 61:526-537.

Vander Zanden, M. J. & Vadeboncoeur, Y. 2002. Fishes as integrators of benthic and pelagic food webs in lakes. *Ecology*, 83:2152-2161.

Vanni, M.J. 2002. Nutrient cycling by animals in freshwater ecosystems. *Annual Review of Ecology and Systematics,* 33:341-370.

Wehr, J.D. & Sheath, R.G. 2003. Freshwater algae of North America: ecology and classification. Maryland Heights, MO: Academic Press.

Table 1. Analysis of Variance results comparing area-specific alkaline phosphatase activity of Lake Tanganyika periphyton between treatment and site in the 2010 fish grazing field experiment.

	Df	Sum Sq	Mean Sq	F Value	p
Treatment	1	0.02313	0.023127	0.6880	0.4218
Site	1	0.00073	0.000728	0.0217	0.8853
Treatment x Site	1	0.01174	0.011743	0.3493	0.5646
Residuals	13	0.43699	0.033614		

Table 2. Analysis of Variance results comparing area-specific alkaline phosphatase activity of Lake Tanganyika periphyton between treatment and site in the 2011 fish grazing laboratory experiment. Significant results indicated by an asterisk.

	Df	Sum Sq	Mean Sq	F Value	p
Treatment	1	89688	89688	0.0197	0.88869
Tank	1	178796	178796871	39.3475	<0.0001*
Treatment x Tank	1	0. 13241070	13241070	2.9139	0.09238
Residuals	68	308995089	4544045		

Table 3. Analysis of Variance results comparing area-specific phosphorous (33P) uptake of Lake Tanganyika periphyton between treatment and site in the 2011 fish grazing laboratory experiment.

	Df	Sum Sq	Mean Sq	F Value	p
Treatment	1	45024	45024	0.0313	0.8604
Tank	1	1882066	1882066	1.3086	0.2591
Treatment x Tank	1	353920	353920	0.2461	0.6224
Residuals	42	60405886	1438235		

Figure 1. Area-specific alkaline phosphatase activity of sites with high (2) and moderate (5 & 6) fish densities in Lake Tanganyika. No significant differences were observed between treatments (p-value= >0.05) or among sites (p-value= >0.05).

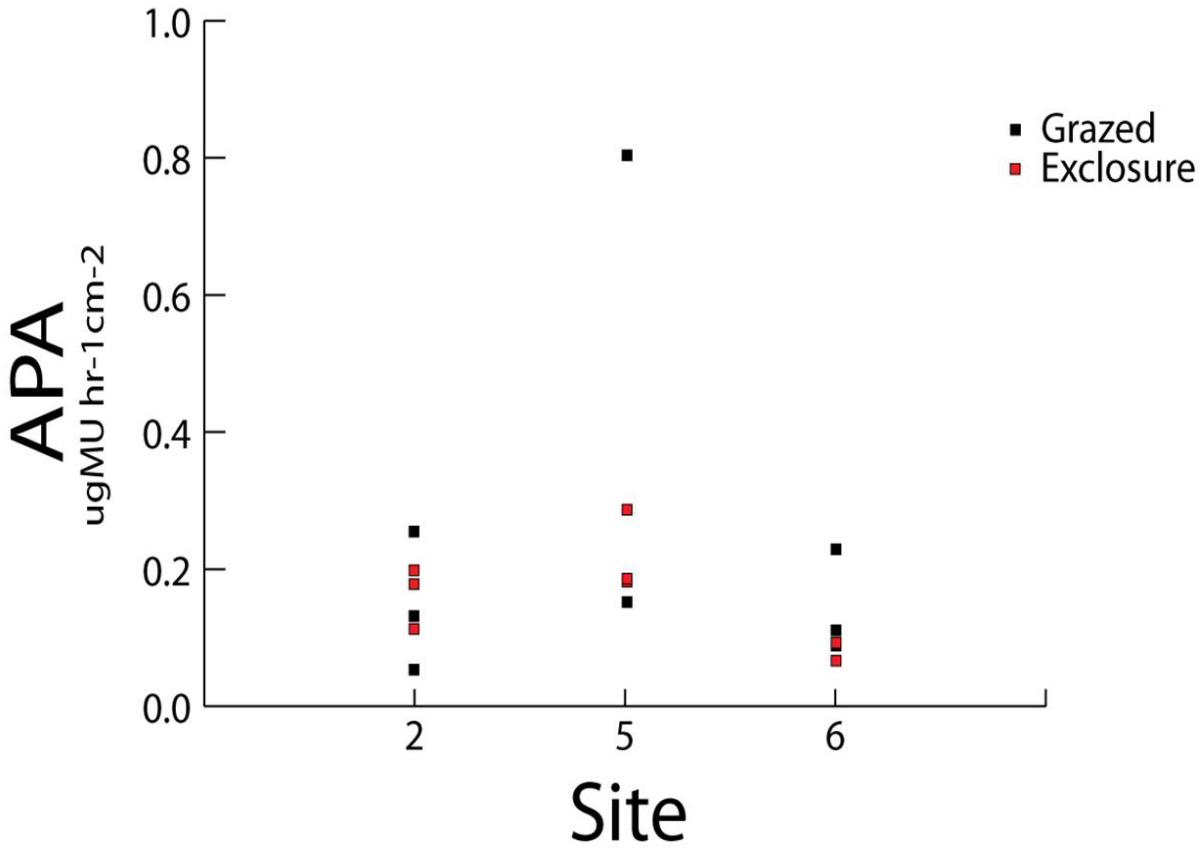


Figure 2. Average area-specific alkaline phosphatase activity from the 2011 laboratory grazing experiment. Error bars represent standard error. The black bars represent the grazed (control) treatment and the grey bars represent the exclosure (experimental) treatment. No significant differences were observed between treatments (p-value= >0.05). The tank containing 7.60g fish mass had significantly lower area-specific alkaline phosphatase activity (p-value= 0.05).

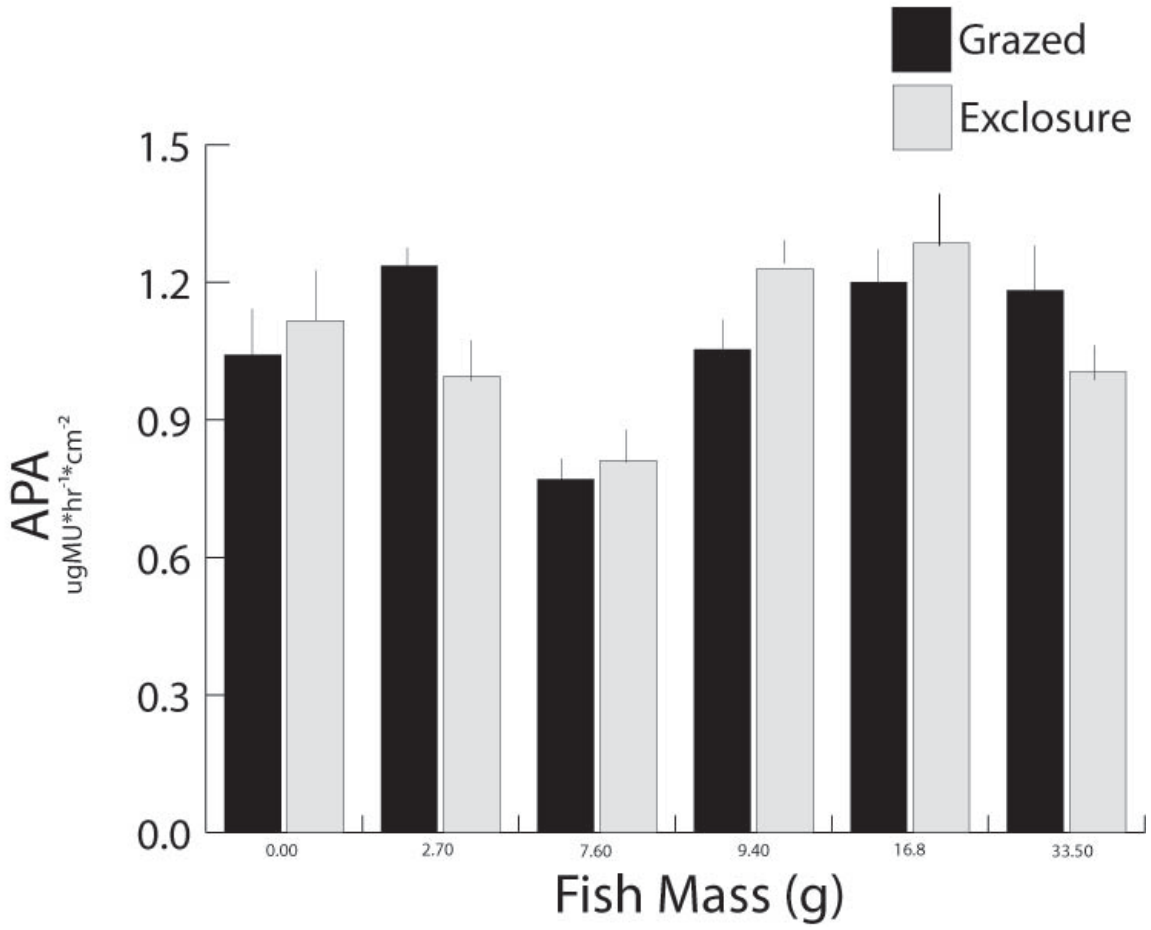


Figure 2. Average area-specific phosphorous uptake (33P) from the 2011 laboratory experiment. Error bars represent standard error. No significant differences were observed between treatments (p-value= >0.05) or among tanks (p-value= >0.05).

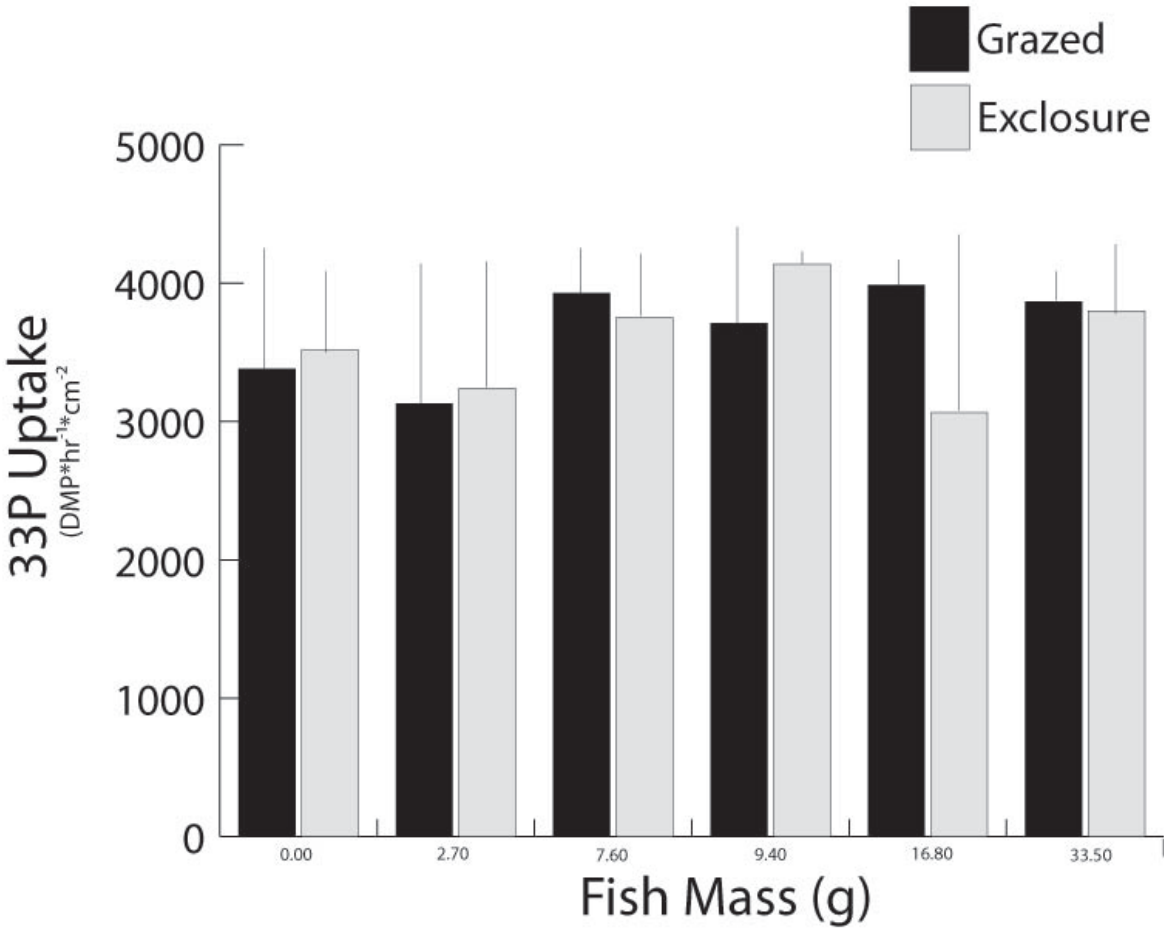


Figure 4. Average area-specific alkaline phosphatase activity for the 12 study sites as part of the 2010 field season. The gray symbols represent the first round of sampling and the black symbols represent the second round of sampling. Error bars represent standard error.